IN THE CLAIMS

111. (Twice Amended) A method of producing a humanized immunoglobulin which specifically binds to a predetermined antigen, the method comprising:

providing a cell containing DNA segments encoding humanized light and heavy chain variable regions; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin;

wherein the cell containing the DNA segments was produced by:

(1) comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions;

(2) selecting a human heavy chain variable region from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region, wherein the selected variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering;

(3) synthesizing the DNA segment encoding the humanized heavy chain variable region, comprising complementarity determining regions (CDRs) from the donor immunoglobulin heavy chain variable region and a variable region framework from the selected acceptor heavy chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin heavy chain variable region and the DNA segment encoding the humanized immunoglobulin light chain variable region into the cell,

wherein the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acid residues identical to those in the acceptor immunoglobulin heavy chain variable region framework.

112. (Amended) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity

determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, and the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

62

~~113.~~ 3 (Amended) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having [complementarity determining regions (CDRs)] hypervariable regions from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the [Kabat and Chothia CDRs] hypervariable regions that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences;

expressing the DNA segments in the cell to produce the humanized immunoglobulin.

166

4 114. (Twice Amended) A method of producing a humanized immunoglobulin having hypervariable regions and framework regions, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of $10^8 M^{-1}$ to $10^{10} M^{-1}$], wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the [Kabat and Chothia CDRs] hypervariable regions that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs; and

expressing the DNA segments in the cell to produce the immunoglobulin.

62

5 115. (Twice Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein the humanized immunoglobulin was produced by:

(1) comparing the sequence of a donor immunoglobulin heavy chain variable region against a collection of sequences of human heavy chain variable regions;

(2) selecting a human heavy chain variable region from the collection of human heavy chain variable regions to provide an acceptor heavy chain variable region, wherein the selected variable region framework is at least 65% identical to the donor immunoglobulin heavy chain variable region framework [and comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework], wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering;

167

(3) synthesizing a DNA segment encoding a humanized heavy chain variable region, comprising complementarity determining regions (CDRs) from the donor immunoglobulin heavy chain variable region and a variable region framework from the selected acceptor heavy chain variable region;

(4) introducing the DNA segment encoding the humanized immunoglobulin heavy chain variable region and a DNA segment encoding a humanized immunoglobulin light chain variable region into a cell; and

(5) expressing the DNA segments in the cell to produce the humanized immunoglobulin,

wherein the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acid residues identical to those in the acceptor immunoglobulin heavy chain variable region framework.

6 ~~16~~. (Twice Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein the humanized immunoglobulin was produced by a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 65% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, and the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acid residues identical to those in the acceptor human immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

168

7 ~~117~~. (Twice Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition, wherein the humanized antibody has complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences,

with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

8 ~~118~~. (Twice Amended) A method of producing a pharmaceutical composition, comprising:

formulating a humanized immunoglobulin with a carrier to form a pharmaceutical composition,

wherein the humanized antibody has complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}], wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs,

with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

9 ~~119~~. (Twice Amended) A humanized immunoglobulin having [complementarity determining regions (CDRs)] hypervariable regions from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the [Kabat and Chothia CDRs] hypervariable regions that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, [,]

wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

10 ~~120~~. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of about 10^8 M^{-1} to 10^{10} M^{-1}], wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the [Kabat and Chothia CDRs] hypervariable regions that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences,

wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

11 ~~121~~. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}], wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin framework outside the Kabat and Chothia CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy or light chain frameworks, and each of these said donor amino acids[:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II)] is capable of interacting with the CDRs,

wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

12 ~~122~~. (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs,

with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

171

13 ~~123.~~ (Twice Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}], wherein said humanized immunoglobulin comprises amino acids from the donor heavy chain immunoglobulin framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) [(II)] is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences,

with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

14 ~~124.~~ A humanized immunoglobulin according to claim ~~120~~ 10, wherein said donor amino acids are from the donor immunoglobulin heavy chain framework.

15 ~~125.~~ A humanized immunoglobulin according to claim ~~121~~ 11, wherein said donor amino acids are from the donor immunoglobulin heavy chain framework.

16 ~~126.~~ A humanized immunoglobulin according to claim ~~122 or 123~~ 12, wherein said humanized immunoglobulin is an antibody tetramer, Fab, or $(Fab')_2$.

17 ~~131.~~ A humanized immunoglobulin according to any one of claims ~~119~~ 9, ~~121~~ 11, or ~~122~~ 12, further comprising an amino acid from the donor immunoglobulin framework that replaces the corresponding amino acid in the acceptor immunoglobulin heavy or light chain frameworks, wherein said amino acid is typical at its position in human immunoglobulin sequences and said corresponding amino acid in the acceptor immunoglobulin is rare at its position in human immunoglobulin sequences.

172

~~132.~~ A humanized immunoglobulin according to any one of claims ~~119~~ to ~~126~~, which comprises at least 3 said amino acids from the donor immunoglobulin framework.

~~133.~~ A humanized immunoglobulin according to any one of claims ~~119~~ to ~~126~~, which comprises two light chain/heavy chain dimers.

~~134.~~ A humanized immunoglobulin according to any one of claims ~~119~~ to [through] ~~126~~, which is substantially pure.

~~135.~~ A pharmaceutical composition comprising a humanized immunoglobulin according to claim ~~134~~ in a pharmaceutically acceptable carrier.

~~136.~~ (Amended) A humanized immunoglobulin according to any one of claims ~~119~~ to [-] ~~126~~, wherein said humanized immunoglobulin has an affinity for antigen [up to about 10^{10} M^{-1}], wherein said affinity is greater than the affinity of another immunoglobulin which has the same sequence as the humanized immunoglobulin except without the donor immunoglobulin framework amino acids that replace the corresponding amino acids in the acceptor immunoglobulin.

~~137.~~ (Amended) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of 10^{8} M^{-1} to 10^{10} M^{-1}], wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the

corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is not outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs, or

(III) is typical at its position for human immunoglobulin sequences, and the replaced amino acid is rare at its position for human immunoglobulin sequences; and

expressing the DNA segments in the cell to produce the humanized immunoglobulin, with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

24 ~~138~~. (Amended) A method of producing a humanized immunoglobulin, the method comprising:

providing a cell containing DNA segments encoding heavy and light chain variable regions of a humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from acceptor immunoglobulin heavy and light chains, which humanized immunoglobulin specifically binds to an antigen with an affinity constant of at least about 10^8 M^{-1} and no greater than about four-fold that of the donor immunoglobulin, wherein said humanized immunoglobulin comprises amino acids from the donor immunoglobulin heavy chain framework outside the Kabat CDRs that replace the corresponding amino acids in the acceptor immunoglobulin heavy chain framework, and each of these said donor amino acids [is outside Chothia CDR H1 (amino acids 26-32) and]:

(I) is adjacent to a CDR in the donor immunoglobulin sequence, or

(II) is capable of interacting with the CDRs;

and expressing the DNA segments in the cell to produce the immunoglobulin, with the proviso that each of these said donor amino acids is outside Chothia CDR H1 (amino acids 26-32).

25 ~~139~~. (Amended) A method of claim 2 ~~112~~, wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable

region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

~~140.~~ 24 (Amended) A method of claim ~~115~~, 5 wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

~~141.~~ 27 (Amended) A method of claim ~~116~~, 6 wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

63

~~142.~~ 28 (Amended) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light chain frameworks, which humanized immunoglobulin specifically binds to an antigen [with an affinity constant of 10^8 M^{-1} to 10^{10} M^{-1}], wherein the sequence of the [humanized] acceptor immunoglobulin heavy chain variable region framework is at least 70% identical to the sequence of the donor immunoglobulin heavy chain variable region framework, and the humanized immunoglobulin heavy chain variable region framework comprises at least 70 amino acids identical to [an] those in the acceptor human immunoglobulin heavy chain variable region [amino acids sequence] framework, wherein percentage sequence identity is determined by aligning amino acids in said frameworks by Kabat numbering.

Please add the following claims:

64

143. (New) A humanized immunoglobulin having complementarity determining regions (CDRs) from a donor immunoglobulin and heavy and light chain variable region frameworks from human acceptor immunoglobulin heavy and light

506 42

175